# Comparative Phytotoxicity Among Four Arsenical Herbicides'

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Abstract. Cacodylic acid (hydroxydimethylarsine oxide) was more phytotoxic than monsodium methanearsonate (MSMA), sodium arsenate, or sodium arsenite when foliarly-applied. MSMA was much more effective on dicotyledonous than on monocotyledonous species. Sodium arsenite and arsenate had little effect on grasses. A comparative study of absorption, transport, and metabolism in beans (*Phaseolus vulgaris* L. 'Black Valentine') revealed that cacodylic acid and MSMA were transported about equally from the leaves to the terminal bud and expanding leaves whereas negligible amounts of sodium arsenite and arsenate were translocated. The latter two compounds caused more rapid contact injury to the treated leaves than either organic arsenical. There was no indication that cacodylic acid or MSMA was demethylated to form inorganic arsenicals or reduced to trivalent arsenic compounds. Studies with "C-MSMA indicated that about 40% of the $^{14}C$ and arsenic recovered was bound rapidly to another molecule to form a ninhydrin-positive complex. In small amounts, arsenate combined with some component of plant tissues. Also, arsenite probably was oxidized to arsenate. In beans, root-applied sodium arsenite was more phytotoxic than sodium arsenate and both were much more phytotoxic than cacodylic acid and MSMA. Most differences in phytotoxicity could not be explained by differences in rates of absorption by bean roots. Arsenite caused considerable contact injury to the root system, probably accounting for its relatively great phytotoxicity. Both cacodylic acid and MSMA were more phytotoxic per mole of tissue arsenic when foliarly-applied than when root-applied.

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## INTRODUCTION

ARSENICALS, inorganic and organic, have been used to control certain forms of cancer and syphilis and as trypanocides, amebicides, insecticides, and herbicides (9, 21). Although they have been studied for many decades, the modes of action of arsenicals in different organisms are not well understood and subject to many interpretations (21). Species selectivity, whether in plants or animals, is a particularly difficult problem, since many arsenicals inhibit respiration or oxidative phosphorylation or inactivate sulfhydryl enzymes, which are fundamental to all organisms. Selectivity aside, several hypotheses concerning the effect of different kinds of arsenicals have been proposed that involve differences in rates of absorption, transport, metabolic conversion of the applied ar-

senical to other more (or less) toxic compounds, or 3 shunt mechanism in resistant organisms (2, 7, 8, 12, 14, 17, 18, 19, 21).

The earliest arsenical herbicide used was sodium arsenite (or arsenic trioxide dissolved in sodium hydroxide); it proved to be an excellent soil sterilizer, although it could be used for selective weed control (4). Sodium, calcium, and lead arsenates have since been used for preemergence weed control in grasses (11). Most recently three organic arsenicals, MSMA, disodium methanearsonate (DSMA), and cacodylic acid, have been used extensively for selective and general weed control. MSMA and DSMA are particularly valuable as selective, preemergence and postemergence herbicides in cotton *(Gossyplum hirsatum* L.) and turf. Cacodylic acid is used for general weed control and is an excellent herbicide for monocotyledonous weeds. Skogley and Ahlgren (16) concluded that cacodylic acid was a more potent soil sterilizer than MSMA, DSMA, or sodium arsenite.

Since arsenicals are valuable herbicides, considerable attention has been given to dosage, time of application, and residual effects in the soil (4, 6, 11, 15); a few studies have correlated herbicidal activity with factors affecting absorption and transport (1, 3, 12). The literature contains relatively few references to comparative herbicidal studies examining both inorganic and organic arsenicals (11, 12, 15, 16) and none comparing activity with application to the leaves and roots. This paper reports the results of investigations on comparative activity with root and foliar applications and also on relative absorption, transport, and metabolism of sodium arsenite, sodium arsenate, MSMA, and cacodylic acid.

## MATERIALS AND METHODS

Seven-day-old 'Black Valentine' bean plants with the primary leaves fully expanded and the first trifoliolate leaf half-expanded were used for root and leaf absorption studies. The plants were grown in artificially-lighted, temperature-controlled chambers at 25 ± 2 C and 60 ± 10% relative humidity with a 16-hr photoperiod. Light intensity was about 14,000 lumens/m2 at plant tops provided by a mixture of fluorescent and incandescent lamps. The plants were immersed in aerated half-strength Hoagland's solution in plastic containers of approximately 1-L capacity. Four to six plants were used for each treatment. Foliar applications were made by micropipet to the upper surface of each primary leaf; five 10-μL droplets were applied to each leaf. Applications to the root system were made by adding measured amounts of arsenicals from stock solutions to give the required concentration when the container was brought to full volume. Initial and final height measurements were made to compute stem elongation during the experimental period. For root applications, the stems were severed above the container level, the roots rinsed thoroughly in large volumes of tap water, and both the roots and shoots dried overnight in a 70 C oven. In separate tests with control plants dipped in arsenical solutions of concentrations equivalent to those used in the experiments, the root rinsing procedure removed all but 0.5 to 5% of the arsenic that was absorbed in a 7-day period. For foliar applications, the treated primary leaves were severed at the first node just below the axillary buds. Thus the plant was divided into three portions: treated leaves, the shoot system above the treated leaves, and the stem and root system below the treated leaves. Each portion was dried as above. Dry weights were recorded and the samples analyzed for arsenic content by methods described elsewhere (13). All values in the tables are corrected for apparent arsenic of the untreated plants: the background values were 0.6 ppm for the stem and leaf tissues and 0.3 ppm for the root system.

Comparative phytotoxicity of spray applications was measured on soil-grown plants raised and treated in a greenhouse in which the minimum temperature was approximately 20 C. Applications were made approximately 7 days after emergence to two 15-cm pots of each of the following species thinned to two plants per pot. 'Black Valentine' bean, soybean *(Glycine max.* (L.) Merr. 'Lincoln'), ivyleaf morningglory *(Ipomea hederacea* (L.) Jacq. 'Heavenly Blue'), radish *(Rhaphanus sativus* (L.) 'Scarlet Globe'). Oats *(Avena sativa* (L.) 'Clinton') and rice *(Oryza sativa* (L.) 'Colusa'), planted thickly, about 30 seed per 15-cm pot, also were treated. Equimolar, aqueous solutions containing 0.5% surfactant (Atplus 401) were applied with a glass atomizer at an operating air pressure of 140 to 350 g/sq cm. Visual observations were made on all treatments 1, 2, 4, and 7 days after treatment. On the seventh day the plants were harvested, dried, and weighed. The maximum inhibition attainable was about 90% since the initial dry weight of the plants was not substracteci from the final value. There was an approximate lo-fold increase in dry weight in the control plants during the 7 days following treatment.

For chromatographic analyses of the treated plants, the dried material was extracted with hot water and the extract filtered and reduced in volume as described elsewhere (13). All chromatography was with Whatman 3MM paper and a descending technique in sealed glass tanks. Solvents used were reagent grade chemicals. Paper strips were eluted with 50% methanol.

Each experiment was repeated, but only the data from one experiment is recorded in the tables and figures.

Sodium arsenite, sodium arsenate, and cacodylic acid were reagent grade crystalline chemicals. MSMA was supplied as a 58% solution. Chromatographic analysis of sodium arsenite and arsenate using four solvent systems revealed no other arsenic-containing compounds. There was some arsenate (up to 1% of the stock solution) in the cacodylic acid and MSMA samples.

Radioactivity was measured with a Nuclear-Chicago Mark I liquid scintillation counter and a Tracer Lab GM, gas-flow, $4\pi$ strip-scanner. The scintillation medium used for counting aqueous solutions contained 10 g of 2,5-diphenyloxazole (PPO) and 80 g of naphthalene per liter of dioxane. Paper strips were counted in a toluene-based scintillation medium containing 0.4% PPO and 0.005% POPOP (*p*-bis[2-(5-phenyloxazolyl)] benzene).

## RESULTS

*Phytotoxicity from foliar applications.* Equimolar applications at 5 X $10^{-3}$M and 1 X $10^{-2}$M showed that, with respect to dry weight inhibition, cacodylic acid was at least as effective as sodium arsenite on the broadleaf

species and considerably more effective on the grasses (Table 1). Sodium arsenite at 5 X $10^{-3}$M caused rapid necrosis on leaves; advanced symptoms were visible 24 hr after treatment; and tip die-back in 'Black Valentine' beans and soybeans occurred within 3 to 4 days. Cacodylic acid-treated dicotyledons revealed marginal leaf burn at 24 hr and severe stunting and tip die-back at 3 to 4 days. In general the cacodylic acid-induced phytotoxicity appeared progressive; whereas that caused by sodium arsenite was immediate, and some plants probably would have survived beyond the 'I-day evaluation period. The only visible effect of cacodylic acid-induced inhibition on oats and rice was severe stunting. Cacodylic acid was clearly superior to MSMA on all but oats and radish, where both were highly effective inhibitors of growth. However, observations revealed that the cacodylic acid-treated radish plants were dead or dying, whereas the MSMA-treated plants would have survived beyond the 7-day experimental period. Sodium arsenate was the least effective compound, although on soybeans at 1 X $10^{-2}$M it caused inhibition of dry-weight gain equal to that caused by cacodylic acid and sodium arsenite. Again, observation showed that the arsenate-treated plants would have survived to maturity whereas the arsenite and cacodylic acid-treated plants were dead or dying after 7 days.

*Foliar absorption and transport.* Five droplets of the arsenicals containing 7.5 μg per droplet were applied to each of the primary leaves of 'Black Valentine' beans. Within 12 to 24 hr after application, necrotic lesions began to appear under the sodium arsenite and arsenate droplets, and some alteration in appearance of the leaf tissue was visible under the MSMA but not under the cacodylic acid droplets. Hence, rinsing the leaves probably would have removed more of the absorbed inorganic arsenicals and MSMA than cacodylic acid, thereby making comparative absorption studies impossible. Three to 7 days after treatment even the cacodylic acid-treated leaves developed lesions. For this reason only the amount of arsenic transported above and below the treated leaves was measured.

The data in Table 2 for 3-day and 7-day transport show clearly that MSMA and cacodylic acid were translocated at substantially greater rates than sodium arsenite or arsenate. These differences were reflected in the greater inhibition of stem elongation by the organic arsenicals. MSMA was translocated more rapidly than cacodylic acid to the root system and probably also to the shoot tip, yet it was not more phytotoxic. If terminal bud death is considered, cacodylic acid was considerably more phytotoxic per mole of arsenite transported than MSMA (Table 2). It appears that between 0.5 and 5 ppm of cacodylic acid in the tissues above the treated leaves was the level required for inhibition; for MSMA the level was between 1 and 7 ppm. At 7 days neither cacodylic acid nor MSMA affected the root system even though root tissue concentrations were almost the same as for the shoot tissues.

Approximately 90,000 dpm of $^{14}$C-MSMA were applied in ten 5-μL droplets to the primary leaves of 'Black Valentine' beans. These smaller droplets did not coalesce and dried about 1 hr after application. Three days after treatment the plants were divided into treated leaves, tissue above treated leaves, and tissue below treated leaves. The three portions were extracted with hot water and treated as described elsewhere (13). The nutrient solutions were evaporated to dryness, and the residue was suspended in a scintillation medium and counted. Samples of the plant tissue extracts were counted; the average value for four plants and the nutrient solution appear in Table 3. The values for transport of $^{14}$C-MSMA were somewhat below that found for the unlabeled material, but the differences may not be statistically significant. Six percent of the $^{14}$C applied was translocated, in approximately the same amounts above and below the treated leaves. A small, but significant, amount of $^{14}$C was found in the nutrient solutions.

***Table*** 1. Percent inhibition of four arsenical herbicides applied as foliar sprays to six plants.

| Plant | Cacodylic acid | | MSMA | | Sodium arsenate | | Sodium arsenite | |
|---|---|---|---|---|---|---|---|---|
| | $10^{-2}$M | 5 X $10^{-3}$M | $10^{-2}$M | 5 X $10^{-3}$M | $10^{-2}$M | 5 X $10^{-3}$M | $10^{-2}$M | 5 X $10^{-3}$M |
| | (%) | (%) | (%) | (%) | (%) | (%) | (%) | (%) |
| Black Valentine beans | 79[a] | 65 | 49 | 48 | 51 | 40 | 79 | 80 |
| Soybeans | 62 | 54 | 41 | 9 | 64 | 23 | 65 | 54 |
| Ivyleaf morningglory | 78 | 46 | 52 | 19 | 11 | 19 | 85 | 20 |
| Radish | 89 | 64 | 72 | 58 | 24 | 7 | 89 | 9 |
| Oats | 71 | 27 | 52 | 36 | 12 | 0 | 39 | 8 |
| Rice | 64 | 74 | 33 | 43 | 37 | 22 | 36 | 30 |

*Percent inhibition is $100 \cdot \frac{\text{Dry weight of treated plants}}{\text{Dry weight of control plants}} \times 100$

***Table*** 2. Transport of arsenicals from 'Black Valentine' bean leaves.'

| Herbicide | Above treated leaf[b] | | Below treated leaf[b] | | Inhibition of stem elongation | | Terminal bud death |
|---|---|---|---|---|---|---|---|
| | 3 days | 7 days | 3 days | 7 days | 3 days | 7 days | 7 days |
| | (ppm) | (ppm) | (ppm) | (ppm) | (%) | (%) | (%) |
| Cacodylic acid,, | 0.53 | 4.6 | 0.28 | 2.0 | 0 | 73 | 75 |
| MSMA | 1.16 | 7.4 | 1.75 | 7.8 | 0 | 76 | 0 |
| Sodium arsenite | 0.03 | 0.6 | 0.00 | 1.1 | 0 | 25 | 0 |
| Sodium arsenate | 0.00 | 0.9 | 0.00 | 1.3 | 0 | 19 | 0 |
| L S D 0 . 0 5 . | 1.1 | 3.0 | 0.4 | 1.3 | — | 19 | |

[a]Seventy-five μg of arsenic as the parent arsenical were applied to each plant.
[b]Each value is the average for four plants. The ppm value is the μg of arsenic divided by the dry weight (g) of the tissue.

*Table* 3. Translocation of "C-MSMA from 'Black Valentine' bean leaves 72 hr after application to leaves.

| Plant fraction or nutrient solution | Radioactivity[a] | Total recovered |
|---|---|---|
| | (dpm) | (%) |
| Treated leaves. | 56,490 | 94 |
| Above treated leaves. | 1,592 | 2.65 |
| Below treated leaves. | 1,878 | 3.13 |
| Nutrient solution. | 112 | 0.19 |

[a]Disintegrations per minute were computed from quench correction curves.

The low values for transport of arsenite were in agreement with the findings of Rumberg *et* al. (12) for soybeans. Arsenate was translocated in approximately the same amounts as arsenite. Thus, the herbicidal activity of these two arsenicals was probably the result of contact damage only.

*Phytotoxicity from root applications.* Equimolar applications of arsenicals to the root systems were not feasible because of the relatively high phytotoxicity of sodium arsenite and low phytotoxicities of cacodylic acid and MSMA. All values for percent inhibition and arsenical concentration in plant tissues were adjusted for differences in initial concentration of applied arsenical in the nutrient solution; thus, for comparing phytotoxicity of root-applied arsenicals the parameter, percent inhibition based on dry weight divided by external concentration, was computed (Figure 1). If the values for 7 days' absorption were used, sodium arsenite was approximately twice as effective as sodium arsenate, seven times more effective than MSMA, and 25 times more effective than cacodylic acid. The differences in relative activity between cacodylic acid and MSMA were not statistically significant.

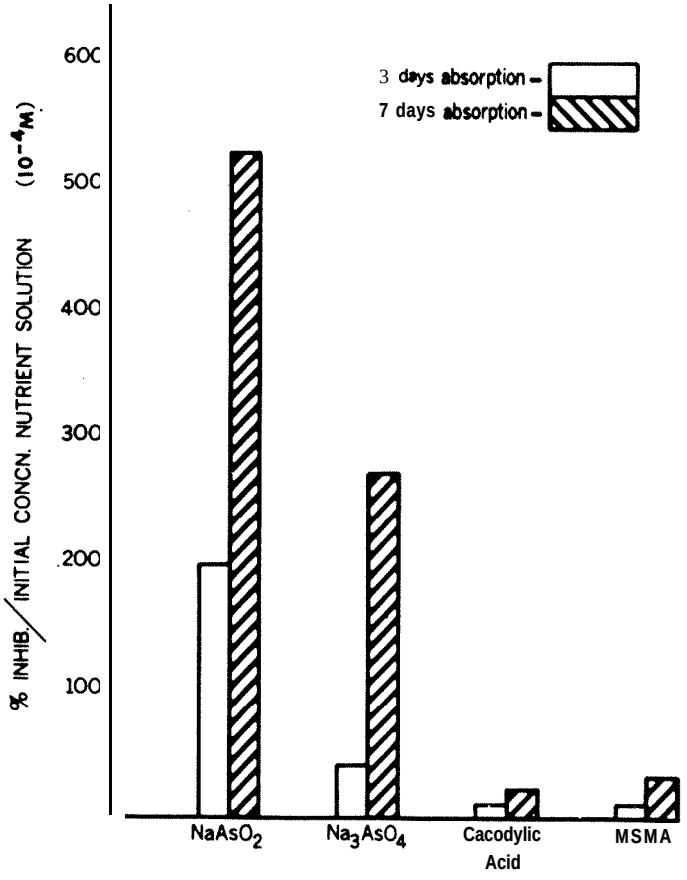


Figure I. Relative effectiveness of root-applied arsenicals on 'Black Valentine' beans after 3 and 7 days' exposure to herbicides in nutrient solutions (see Table 4 for concentrations). Percent inhibition was computed as follows: Percent inhibition $= 100 - \frac{\text{Dry weight of treatment}}{\text{Dry weight of control}} \times 100$.

*Root absorption and transport.* Sodium arsenite and arsenate were more readily absorbed by the root system of 'Black Valentine' beans than either MSMA or cacodylic acid. (Figure 2). The measure of relative absorption was concentration of the arsenical in the plant tissue divided by the initial concentration in the nutrient solution. On this basis sodium arsenate was absorbed 32 times, sodium arsenite 26 times, and MSMA 14 times more readily than cacodylic acid. However, if the ratio of arsenical concentration in the tops to that in the roots can be used as a measure of transport, cacodylic acid was transported to the tops 5 to 10 times more rapidly than MSMA, arsenite, or arsenate (Table 4). As expected, the concentrations of each arsenical in the shoot system after 3 or 7 days' absorption by the roots (Table 4) were higher compared with those observed 3 or 7 days after foliar application (Table 2). Yet only the arsenate and the arsenite-treated plants were inhibited more by root than by foliar application. Root-applied sodium arsenite significantly inhibited the root system whereas little or no inhibition was noted in the other treatments.

*Metabolism of arsenicals.* In cacodylic acid and MSMA-treated plants about 3 to 5% of the arsenic recovered in the extract remained bound to the insoluble residue 7 days after root or foliar applications. Up to 25% of the arsenic recovered remained bound to the residue in arsenite and arsenate-treated plants. No attempt was made to identify the bound arsenic. The extracts were analyzed by paper chromatographic procedures (13). Particular attention was given to the problems of demethylation of cacodylic acid and MSMA, complex formation of cacodylic acid and MSMA, reduction of arsenate to arsenite,

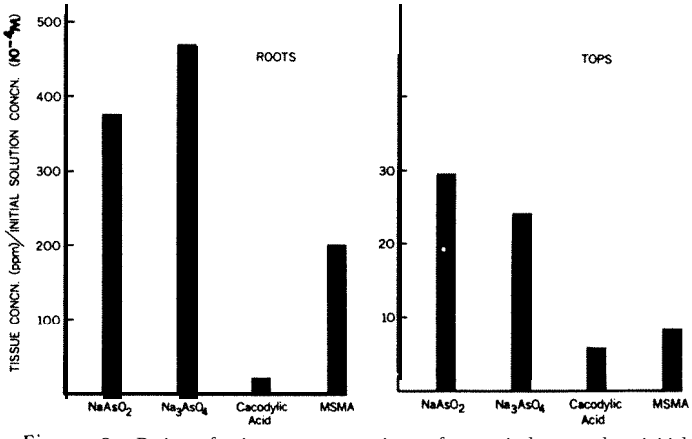


*Figure* 2. Ratio of tissue concentration of arsenicals to the initial concentration in the nutrient solution for 'Black Valentine' beans exposed for 3 days to herbicides in nutrient solution (see Table 4 for concentrations).

Table 4. Absorption and transport of root-applied arsenicals on 'Black Valentine beans harvested 3 and 7 days after exposure to the herbicides in nutrient solution.

| Herbicide | Initial concn in nutrient solution | Arsenic in roots | | Arsenic in tops | | Ratio of arsenic tops/roots | | Inhibition of root growth* |
|---|---|---|---|---|---|---|---|---|
| | | 3 days | 7 days | 3 days | 7 days | 3 days | 7 days | |
| Cacodylic acid | $2 \times 10^{-4}$ M | (ppm) 29 | (ppm) 44 | (ppm) 7 | (ppm) 18 | 0.41 | 0.41 | (%) 10 |
| MSMA | $2 \times 10^{-4}$ | 401 | 393 | 17 | 21 | 0.04 | 0.05 | 0 |
| Sodium arsenite | $2 \times 10^{-5}$ | 75 | 69 | 5 | 5 | 0.07 | 0.07 | 44 |
| Sodium arsenate | $2 \times 10^{-5}$ | 93 | 121 | 4 | 2 | 0.04 | 0.02 | 3 |
| LSD 0.05 | | 86 | 32 | 3 | 6 | | | 30 |

*Percent inhibition = 100 . $\frac{\text{Dry weight of root system for treated plants}}{\text{Dry weight of root system for control plants}}$ x 100

and the formation of organic arsenylated complexes. The solvent systems chosen for analysis of extracts of cacodylic acid-treated plants were 1-propanol:ammonium hydroxide (7:3 v/v) (solvent system I) and 2-propanol: water: acetic acid (10:3:0:1 v/v/v) (solvent system II). Paper chromatograms were cut into strips and divided into 10 $R_f$ units for arsenic analyses; for each run, half to two thirds of the chromatogram, depending upon the amount of arsenic present, was retained for elution of arsenic-positive zones.

The analysis for an extract of cacodylic acid-treated plants is shown in Table 5; the eluate from $R_f$ 0.2 to 0.4, containing most of the arsenic, was rechromatographed in solvent system II. Again, most of the activity was recovered at $R_f$ 0.5 to 0.7, corresponding almost exactly to the expected value for cacodylic acid. Portions of these chromatograms were analyzed for the presence of trivalent arsenic by omitting acid digestion and reductants before generation of nascent hydrogen (13). When this procedure was followed, no arsenic could be detected on the chromatograms. Hence, trivalent arsenic was present at less than 0.1 ppm in the extracts.

In one experiment, bean plants held in an air-tight Plexiglas chamber were treated with $5 \times 10^{-4}$M cacodylic acid added to the nutrient solution. For 5 days after treatment an airstream passed through the nutrient solutions, into the atmosphere surrounding the plant, and then through 5% mercuric bromide in 95% ethanol, a solution that traps arsine and oxidizes it to elemental arsenic. No precipitate was observed. The solution was filtered through Whatman 42 paper (1 μ pore size), and the paper then was analyzed for arsenic. No arsenic was present on the paper. Hence, it can be concluded that arsine gas probably was not liberated during the 5-day experimental period. During this time the four treated plants had developed severe toxicity symptoms and had accumulated in excess of 350 μg of arsenic as cacodylic acid.

*Table* 5. Micrograms of arsenic within 10 $R_f$ ranges after paper chromatographic analysis of extracts of arsenical-treated plants in three solvent systems.*

| $R_f$ range | Cacodylic acid | | MSMA[b] | | | Sodium arsenite | | Sodium arsenate | |
|---|---|---|---|---|---|---|---|---|---|
| | I | II | I | IIA | IIB | I | III | I | III |
| | (μg) | (μg) | (μg) | (μg) | (μg) | (μg) | (μg) | (μg) | (μg) |
| 0–0.1 | 0 | 0.4 | 31 | 2.3 | 0.4 | 17.8 | 0.8 | 13 | 0.8 |
| 0.1–0.2 | 0 | 0 | 7.8 | 1.0 | 0.3 | 0 | 0.4 | 5 | 0 |
| 0.2–0.3 | 6.8 | 0 | 13.5 | 12.0 | 0 | 0 | 3.6 | 0 | 2.9 |
| 0.3–0.4 | 0.9 | 0.1 | 5.6 | 7.0 | 0 | 0 | 3.7 | 0 | 18.9 |
| 0.4–0.5 | 0 | 0.1 | 0 | 4.5 | 0 | 0 | 0 | 0 | 0.9 |
| 0.5–0.6 | 0 | 4.2 | 0 | 3.0 | 0 | 0 | 2.4 | 0 | 0 |
| 0.6–0.7 | 0 | 5.7 | 0 | 0.5 | 0.7 | 0 | 14.0 | 0 | 1.8 |
| 0.7–0.8 | 0 | 0 | 0 | 0.6 | 2.0 | 0 | 0 | 0 | 5.7 |
| 0.8–0.9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.9–1.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*The Roman numerals at the heads of columns identify the solvent systems used for chromatographic analysis as follows:
I was 1-propanol:ammonium hydroxide (7:3 v/v)
II was 2-propanol:water:acetic acid (10:3:0.1 v/v/v)
III was 2-propanol:water (7:3 v/v)
[b]Two zones (Rr 0 to 0.2 and $R_f$ 0.2 to 0.4) were eluted from chromatograms developed in solvent system I and rechromatographed in solvent system III. Columns for these chromatograms are identified as IIA and IIB for zones with $R_f$ of 0 to 0.2 and 0.2 to 0.4, respectively.

Chromatographic analyses of extracts from MSMA-treated plants were made with solvent systems I and II. Two $R_f$ zones were eluted from the chromatogram developed in solvent system I (0 to 0.2 and 0.2 to 0.4) and run in solvent system II. The arsenic recovered from the 0 to 0.2 zone was largely MSMA, since it co-chromatographed with samples of MSMA added to plant extract. However, there were significant amounts of arsenic at the origin and at 0.4 to 0.8 that cannot be explained by "tailing". Analysis of the 0.2 to 0.4 zone indicates that an arsenic compound other than MSMA, running at 0.7 to 0.8, was formed in the bean tissues. In experiments with $^{14}$C-MSMA, extracts of the treated leaves were developed in solvent system II; and two clear peaks of radioactivity, both associated with substantial amounts of arsenic, were resolved (Figure 3). Preliminary studies with solvent system II indicate that the compound moving at 0.35 was MSMA and that moving at 0.69 was probably MSMA complexed with a ninhydrin-positive substance. The activity associated with the ninhydrin-positive substance was about 60% of that on the chromatogram.

Chromatographic analyses of extracts of arsenite and arsenate-treated plants were made with solvent systems I

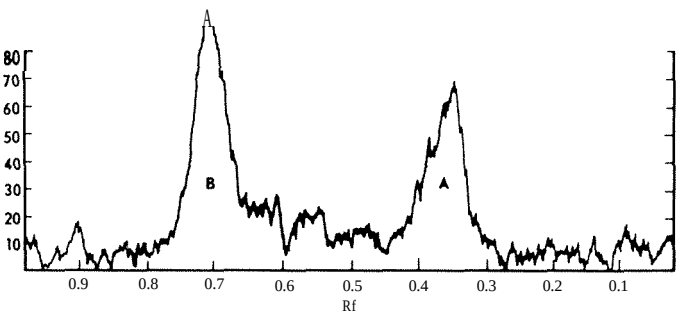


*Figure* 3. Strip scan of a chromatogram of an extract from $^{14}$C-MSMA-treated bean leaves. The solvent system was 2-propanol: water:acetic acid (10:3:0.1 v/v/v). Peak A at $R_f$ 0.35 is presumably $^{14}$C-MSMA; peak B at $R_f$ 0.7 is a ninhydrin-positive, arsenic. containing compound.

and III (2-propanol:water, 7:3 v/v). If plant extracts were added at the origin, arsenite and arsenate remained at 0 to 0.1 $R_f$ units with solvent system I. When eluted and rechromatographed in solvent system III, arsenite moved to 0.6 to 0.7 and arsenate to 0.2 to 0.3. There were certain similarities in the analyses of the extracts (Table 5) which suggest that (a) arsenite was oxidized to form arsenate or it formed an arsenylated compound moving at the same $R_f$ value as arsenate, and (b) arsenate formed an arsenylated compound moving at an $R_f$ value characteristic of a less polar compound than arsenate, arsenite, or MSMA.

## Discussion

Cacodylic acid was the most effective of the four arsenical herbicides when applied to the foliage (Table 1), yet it was the least effective when root-applied to beans (Figure 1). Sodium arsenite was a potent foliarly-applied herbicide on dicotyledonous species, had little effect on oats and rice (Table I), and was by far the most effective arsenical when applied to the roots (Figure 1). To a great extent these differences in relative activity, which were dependent on site of application, could be explained by differences in translocation from treated leaves (cacodylic acid moves whereas arsenite does not) or by root absorption (arsenite is absorbed 25 times more readily than cacodylic acid), but there were differences observed that could be explained only on the basis of differences in intrinsic activity among the arsenicals. For example, MSMA was translocated from bean leaves more rapidly and was present at nearly twice the tissue concentration as cacodylic acid, yet the latter was clearly more phytotoxic. Hence, one concludes that cacodylic acid is a more effective inhibitor of biological processes critical to plant growth.

Cacodylic acid and MSMA were much less effective per unit arsenic in the shoot tissues when applied to the root than when applied to the foliage. Probably the root-applied arsenicals were carried passively in the transpiration stream and, hence, accumulated largely in the primary and trifoliolate leaves rather than in the meristematic tissues of the stem. It appears that the shoot meristematic tissues are the major sites for herbicidal action for cacodylic acid and MSMA.

Neither arsenite nor arsenate were translocated from bean leaves, and both were rapidly absorbed by bean roots; yet arsenite was at least twice as phytotoxic as arsenate whether foliarly or root-applied. Arsenite is a well-known thiol reagent, combining rapidly with dithiol groups on proteins, and, hence, is an effective inhibitor of enzymes requiring free sulfhydryl groups (21). Arsenate, on the other hand, is a competitive inhibitor of phosphate (10) and acts as an uncoupler of oxidative phosphorylation (21), but is not a thiol reagent until it is reduced to the trivalent form. Thus, arsenate-treated plants are inhibited mainly by inadequate levels of phosphorylated compounds, whereas arsenite-treated plants may suffer the immediate loss of activity of vital enzymes, including those necessary for oxidative phosphorylation (21). Moreover, if dithiol groups are required for maintenance of membrane integrity in plants, the very rapid desiccation of leaves and extensive root inhibition observed in arsenite-treated plants was to be expected (Table 4).

Two of the most interesting questions concerning arsenical herbicides are: (a) why is sodium arsenite relatively ineffective on oats and rice and yet so potent on dicotyledonous species, and (b) why are MSMA and cacodylic acid so effective on grasses? To answer these questions, additional studies are required to compare absorption and metabolism of arsenite and cacodylic acid or MSMA in resistant monocotyledonous species with those in sensitive dicotyledonous species. Preliminary studies reveal that cacodylic acid, MSMA, sodium arsenite, and sodium arsenate are absorbed in nearly equal quantities by the root systems of oats and are translocated to the leaves. Only sodium arsenate failed to inhibit growth. Oats probably possess a mechanism for inactivating relatively large amounts of arsenate. Excess tissue phosphate or the failure to concentrate arsenate in meristematic tissues may account for arsenate resistance. The resistance of oats to foliar applications of sodium arsenite was due probably to poor absorption by the leaves and not to metabolic factors.

Since arsenite is such an effective herbicide on dicotyledonous species, it would be of interest to test trivalent organic arsenicals, such as phenyl arsenoxide and related compounds that have been used as tryparnocides, for herbicidal activity on grasses as well as dicotyledonoua species. In general, trivalent arsenicals are much more effective biological agents than the pentavalent compounds (21) and hence the trivalent equivalents of cacodylic acid and MSMA would be most interesting to test.

Cacodylic acid was translocated to the shoot system 6 to IO-times more readily than any other arsenical tested. This is a particularly important factor in comparing activity of soil-applied herbicides, and may account in part for the greater soil sterilant activity of cacodylic acid compared with MSMA, sodium arsenite, and other arsenicals (16). Although arsenite was more effective than cacodylic acid and MSMA in solution culture experiments, it may be inactivated in the soil by oxidation or bound rapidly in some insoluble form, perhaps as a calcium salt (21), and thereby lose much of its effectiveness relative to cacodylic acid and MSMA. Soil inactivation has been described by Clements and Munson (3) who found that arsenite was highly phytotoxic in solution culture yet of reduced effectiveness in soils. Also, Duble *et* al. (5) found ready absorption of DSMA from solution culture but not from soils, and Ehman (6) reported rapid inactivation of cacodylic acid and MSMA in soils. Inactivation may be due to bonding to some mineral structure, an ion-exchange phenomenon, or biological degradation (20).

Cacodylic acid was apparently a very stable compound in bean plants; chromatographic analyses revealed little or no arsenic associated with any fraction other than cacodylic acid itself. No arsine gas was detected in experiments in which severe phytotoxic effects were observed on bean plants. Thus, a much discussed hypothesis that phytotoxicity of arsenicals is the result of reduction to volatile arsines has not received support.

MSMA forms a ninhydrin-positive complex in bean plants, which may bear significantly on its phytotoxic action not only in beans but other species as well (5, 14).

Sckerl and Frans (14) postulated that such a complex may block a specific biosynthetic pathway and this blockage may account for the herbicidal activity of MSMA. Indeed, in support of this hypothesis, they observed much higher amino acid levels in treated johnsongrass (*Sorghum halepense* (L.) Pers.), an MSMA-sensitive species, and only slightly altered levels in cotton, a more resistant species. Duble *et al.* (5) found that $^{14}$C-DSMA was readily translocated acropetally and basipetally in spite of the fact that it was complexed almost completely with some component in the plant; hence, the complex and not DSMA was the mobile form of the herbicide. In beans MSMA was less phytotoxic than cacodylic acid and the latter did not form a complex. One might argue that the MSMA-complex may account for reduced, not increased, phytotoxicity in beans. It remains to be shown, preferably in tests with isolated enzyme systems, whether the complex possesses the same or altered phytotoxicity compared with MSMA.

The carbon-arsenic bond in $^{14}$C-MSMA was not broken in bean leaves; in a 3-day experiment with $^{14}$C-MSMA-treated excised leaves in sealed Petri dishes, barium hydioxide contained no $^{14}$C. Duble *et* al. (5) found less than 0.1% of the $^{14}$C- applied as DSMA released as volatile $^{14}$C 10 days after treatment of coastal bermudagrass (*Cynodon dactylon* (L.) Pers.), and they concluded that the carbon-arsenic bond was stable in bermudagrass. In soils, however, Von Endt *et* al. (20) found relatively rapid loss of $^{14}$C from $^{14}$C-MSMA; hence, the carbon-arsenic bond is subject to attack by biological systems.

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